Revision 2

Store at -20°C

#9931

Phospho-Chk1/2 Antibody Sampler Kit

1 Kit
(9 X 20 µL)

Orders n 877-616-CELL (2355)
orders@cellsignal.com
Support n 877-678-TECH (8324)
info@cellsignal.com
Web n www.cellsignal.com

For Research Use Only. Not For Use In Diagnostic Procedures.

Products Included	Product #	Quantity	Mol. Wt.	Isotype
Phospho-Chk1 (Ser317) (D12H3) XP® Rabbit mAb	12302	20 µL	56 kDa	Rabbit IgG
Phospho-Chk1 (Ser345) (133D3) Rabbit mAb	2348	20 µL	56 kDa	Rabbit IgG
Phospho-Chk2 (Ser19) Antibody	2666	20 µL	62 kDa	Rabbit IgG
Phospho-Chk2 (Ser33/35) Antibody	2665	20 µL	62 kDa	Rabbit IgG
Phospho-Chk2 (Thr68) (C13C1) Rabbit mAb	2197	20 µL	62 kDa	Rabbit IgG
Phospho-Chk2 (Ser516) Antibody	2669	20 µL	62 kDa	Rabbit IgG
Phospho-Chk1 (Ser296) Antibody	2349	20 µL	56 kDa	Rabbit IgG
Chk1 (2G1D5) Mouse mAb	2360	20 µL	56 kDa	Mouse IgG1
Chk2 (D9C6) Rabbit mAb	6334	20 µL	62 kDa	Rabbit IgG
Anti-rabbit IgG, HRP-linked Antibody	7074	100 µL		Goat

See www.cellsignal.com for individual component applications, species cross-reactivity, dilutions and additional application protocols.

Description: The Phospho-Chk1/2 Antibody Sampler Kit offers an economical means to evaluate the phosphorylation status of Chk1 and Chk2 on multiple residues. The kit includes enough antibody to perform two western blot experiments with each primary antibody.

Background: Chk1 kinase acts downstream of ATM/ATR kinase and plays an important role in DNA damage checkpoint control, embryonic development, and tumor suppression (1). Activation of Chk1 involves phosphorylation at Ser317 and Ser345 and occurs in response to blocked DNA replication and certain forms of genotoxic stress (2). While phosphoryla-tion at Ser345 serves to localize Chk1 to the nucleus fol-lowing checkpoint activation (3), phosphorylation at Ser317 along with site-specific phosphorylation of PTEN allows for reentry into the cell cycle following stalled DNA replication (4). Chk1 exerts its checkpoint mechanism on the cell cycle, in part, by regulating the cdc25 family of phosphatases. Chk1 phosphorylation of cdc25A targets it for proteolysis and inhibits its activity through 14-3-3 binding (5). Activated Chk1 can inactivate cdc25C via phosphorylation at Ser216, blocking the activation of cdc2 and transition into mitosis (6). Centrosomal Chk1 has been shown to phosphorylate cdc25B and inhibit its activation of CDK1-cyclin B1, thereby abrogat-ing mitotic spindle formation and chromatin condensation (7). Furthermore, Chk1 plays a role in spindle checkpoint function through regulation of Aurora B and BubR1 (8). Research studies have implicated Chk1 as a drug target for cancer therapy as its inhibition leads to cell death in many cancer cell lines (9).

Chk2 is the mammalian homologue of the budding yeast Rad53 and fission yeast Cds1 checkpoint kinases (5-7). The amino-terminal domain of Chk2 contains a series of seven serine or threonine residues (Ser19, Thr26, Ser28, Ser33, Ser35, Ser50 and Thr68) followed by glutamine (SQ or TQ motif). These are known to be preferred sites for phosphorylation by ATM/ATR kinases (8). Indeed, after DNA damage by ionizing radiation (IR), UV irradiation and DNA replication blocked by hydroxyurea, Thr68 and other sites in this region become phosphorylated by ATM/ATR (9-11). The SQ/TQ cluster domain, therefore, seems to have a regulatory function. Phosphorylation at Thr68 is a prerequisite for the subsequent activation step, which is attributable to autophos-phorylation of Chk2 on residues Thr383 and Thr387 in the activation loop of the kinase domain (12).

Specificity/Sensitivity: Each antibody in the Phospho-Chk1/2 Antibody Sampler Kit detects endogenous levels of its respective target protein.

Source/Purification: Polyclonal antibodies are produced by immunizing animals with a synthetic peptide and are purified by protein A and peptide affinity chromatography. Monoclonal antibodies are produced by immunizing animals with recombinant human proteins or synthetic peptides.

Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA and 50% glycerol. Store at –20°C. *Do not aliquot the antibodies.*

Recommended Antibody Dilutions:

Western blotting 1:1000

Please visit www.cellsignal.com for validation data and a complete listing of recommended companion products.

Background References:

(1) Liu, Q. et al. (2000) *Genes Dev* 14, 1448-59.
(2) Zhao, H. and Piwnica-Worms, H. (2001) *Mol Cell Biol* 21, 4129-39.
(3) Jiang, K. et al. (2003) *J Biol Chem* 278, 25207-17.
(4) Martin, S.A. and Ouchi, T. (2008) *Mol Cancer Ther* 7, 2509-16.
(5) Chen, M.S. et al. (2003) *Mol Cell Biol* 23, 7488-97.
(6) Zeng, Y. et al. (1998) *Nature* 395, 507-10.
(7) Löffler, H. et al. (2006) *Cell Cycle* 5, 2543-7.
(8) Zachos, G. et al. (2007) *Dev Cell* 12, 247-60.
(9) Garber, K. (2005) *J Natl Cancer Inst* 97, 1026-8.
(10) Allen, J. B. et al. (1994) *Genes Dev.* 8, 2401-2415.
(11) Weinert, T. A. et al. (1994) *Genes Dev.* 8, 652-665.
(12) Murakami, H. and Okayama, H. (1995) *Nature* 374, 817-819.
(13) Kastan, M.B. and Lim, D.S. (2000) *Nat. Rev. Mol. Cell Biol.* 1, 179-186.
(14) Matsuoka, S. et al. (2000) *Proc. Natl. Acad. Sci. USA* 97, 10389-10394.
(15) Melchionna, R. et al. (2000) *Nat. Cell Biol.* 2, 762-765.
(16) Ahn, J. Y. et al. (2000) *Cancer Res.* 60, 5934-5936.

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Applications Key: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide

Species Cross-Reactivity Key: **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **Ce**—C. elegans **Hr**—Horse **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.

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Western Immunoblotting Protocol

For western blots, incubate membrane with diluted primary antibody in either 5% w/v BSA or nonfat dry milk, 1X TBS, 0.1% Tween® 20 at 4°C with gentle shaking, overnight.
NOTE: Please refer to primary antibody datasheet or product webpage for recommended primary antibody dilution buffer and recommended antibody dilution.

A. Solutions and Reagents

NOTE: Prepare solutions with reverse osmosis deionized (RODI) or equivalent grade water.

1. **20X Phosphate Buffered Saline (PBS):** (#9808) To prepare 1 L 1X PBS: add 50 ml 20X PBS to 950 ml dH_2O, mix.
2. **10X Tris Buffered Saline (TBS):** (#12498) To prepare 1 L 1X TBS: add 100 ml 10X to 900 ml dH_2O, mix.
3. **1X SDS Sample Buffer:** Blue Loading Pack (#7722) or Red Loading Pack (#7723) Prepare fresh 3X reducing loading buffer by adding 1/10 volume 30X DTT to 1 volume of 3X SDS loading buffer. Dilute to 1X with dH_2O.
4. **10X Tris-Glycine SDS Running Buffer:** (#4050) To prepare 1 L 1X running buffer: add 100 ml 10X running buffer to 900 ml dH_2O, mix.
5. **10X Tris-Glycine Transfer Buffer:** (#12539) To prepare 1 L 1X transfer buffer: add 100 ml 10X transfer buffer to 200 ml methanol + 700 ml dH_2O, mix.
6. **10X Tris Buffered Saline with Tween® 20 (TBST):** (#9997) To prepare 1 L 1X TBST: add 100 ml 10X TBST to 900 ml dH_2O, mix.
7. **Nonfat Dry Milk:** (#9999)
8. **Blocking Buffer:** 1X TBST with 5% w/v nonfat dry milk; for 150 ml, add 7.5 g nonfat dry milk to 150 ml 1X TBST and mix well.
9. **Wash Buffer:** (#9997) 1X TBST
10. **Bovine Serum Albumin (BSA):** (#9998)
11. **Primary Antibody Dilution Buffer:** 1X TBST with 5% BSA or 5% nonfat dry milk as indicated on primary antibody datasheet; for 20 ml, add 1.0 g BSA or nonfat dry milk to 20 ml 1X TBST and mix well.
12. **Biotinylated Protein Ladder Detection Pack:** (#7727)
13. **Prestained Protein Marker, Broad Range (Premixed Format):** (#7720)
14. **Blotting Membrane and Paper:** (#12369) This protocol has been optimized for nitrocellulose membranes. Pore size 0.2 µm is generally recommended.
15. **Secondary Antibody Conjugated to HRP:** anti-rabbit (#7074); anti-mouse (#7076)
16. **Detection Reagent:** LumiGLO® chemiluminescent reagent and peroxide (#7003) or SignalFire™ ECL Reagent (#6883)

B. Protein Blotting

A general protocol for sample preparation.

1. Treat cells by adding fresh media containing regulator for desired time.
2. Aspirate media from cultures; wash cells with 1X PBS; aspirate.
3. Lyse cells by adding 1X SDS sample buffer (100 µL per well of 6-well plate or 500 µL for a 10 cm diameter plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
4. Sonicate for 10–15 sec to complete cell lysis and shear DNA (to reduce sample viscosity).
5. Heat a 20 µL sample to 95–100°C for 5 min; cool on ice.
6. Microcentrifuge for 5 min.
7. Load 20 µL onto SDS-PAGE gel (10 cm x 10 cm). **NOTE:** Loading of prestained molecular weight markers (#7720, 10 µL/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 µL/lane) to determine molecular weights are recommended.
8. Electrotransfer to nitrocellulose membrane (#12369).

C. Membrane Blocking and Antibody Incubations

NOTE: Volumes are for 10 cm x 10 cm (100 cm^2) of membrane; for different sized membranes, adjust volumes accordingly.

I. Membrane Blocking

1. (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 min at room temperature.
2. Incubate membrane in 25 ml of blocking buffer for 1 hr at room temperature.
3. Wash three times for 5 min each with 15 ml of TBST.

II. Primary Antibody Incubation

1. Incubate membrane and primary antibody (at the appropriate dilution and diluent as recommended in the product datasheet) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
2. Wash three times for 5 min each with 15 ml of TBST.
3. Incubate membrane with the species appropriate HRP-conjugated secondary antibody (#7074 or #7076 at 1:2000) and anti-biotin, HRP-linked Antibody (#7075 at 1:1000–1:3000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hr at room temperature.
4. Wash three times for 5 min each with 15 ml of TBST.
5. Proceed with detection (Section D).

D. Detection of Proteins

1. Incubate membrane with 10 ml LumiGLO® (0.5 ml 20X LumiGLO® #7003, 0.5 ml 20X peroxide, and 9.0 ml purified water) or 10 ml SignalFire™ #6883 (5 ml Reagent A, 5 ml Reagent B) with gentle agitation for 1 min at room temperature.
2. Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10 sec exposure should indicate the proper exposure time. **NOTE:** Due to the kinetics of the detection reaction, signal is most intense immediately following incubation and declines over the following 2 hr.

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